PB84-208107

Relative Sensitivity of 'Chironomus tentans' Life Stages to Copper

(U.S.) Corvallis Environmental Research Lab., OR

1984

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/J-84-031	2.
3. RE PB84-208107	
4. TITLE AND SUBTITLE: Relative Sensitivity of Chironomus tentans Life Stages to Copper (Journal Version)	5. REPORT DATE: 1984
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): Alan V. Nebeker, Michael A. Cairns and Claudia M.Wise	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Environmental Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Corvallis, Oregon 97333	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS: same	13. TYPE OF REPORT AND PERIOD COVERED: journal article
	14. SPONSORING AGENCY CODE: EPA/600/02

15. SUPPLEMENTARY NOTES

Published in: Environmental Toxicology and Chemistry 3:151-158, 1984

16. ABSTRACT

The four larval instars of the midge Chironomus tentans Fabricius were exposed to copper to determine their relative sensitivities. The impact of copper on adult emergence and effect of exposure time on LC50 values were also determined. First-instar larvae appeared to be the most sensitive to acute exposure, with a 96-h LC50 of 298 µg/L copper, followed by second-instar (LC50=773 µg/L), third-instar (LC50=1690 µg/L) larvae, at a water hardness of 71 to 84 mg/L. Adults emerged successfully from fourth-instar larvae and pupae that survived 20-D copper exposures of up to 235 µg/L; the 20-day EC50 was 77.5 µg/L.

Methods for continuous culture of C. tentans in a flow-through rearing facility using Cerophyl, a commercially available powdered grass product, as food and substrate are presented.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT: Release to public	19. SECURITY CLASS *(This Report)*: unclassified	21. NO. OF PAGES: 9
	20. SECURITY CLASS *(This page)*: unclassified	22. PRICE

EPA Form 2220-1 (9-73)

EPA-600/J-84-031
JOURNAL ARTICLE

Environmental Toxicology and Chemistry, Vol. 3, pp. 151-158, 1984
Printed in the USA. Pergamon Press Ltd.
0730-7268/84/$3.00 + .00
1984 SETAC

RELATIVE SENSITIVITY OF *CHIRONOMUS TENTANS* LIFE STAGES TO COPPER

ALAN V. NEBEKER,* MICHAEL A. CAIRNS and CLAUDIA M. WISE

U.S. Environmental Protection Agency
Corvallis Environmental Research Laboratory
Western Fish Toxicology Station
Corvallis, Oregon 97333

(*Received* 13 *May* 1983; *Accepted* 28 *September* 1983)

Abstract — The four larval instars of the midge *Chironomus tentans* Fabricius were exposed to copper to determine their relative sensitivities. The impact of copper on adult emergence and effect of exposure time on LC_{50} values were also determined. First-instar larvae appeared to be the most sensitive to acute exposure, with a 96-h LC_{50} of 298 μg/L copper, followed by second-instar (LC_{50} = 773 μg/L), third-instar (LC_{50} = 1,446 μg/L) and fourth-instar (LC_{50} = 1,690 μg/L) larvae, at a water hardness of 71 to 84 mg/L. Adults emerged successfully from fourth-instar larvae and pupae that survived 20-d copper exposures of up to 235 μg/L; the 20-day EC_{50} was 77.5 μg/L.

Methods for continuous culture of *C. tentans* in a flow-through rearing facility using Cerophyl, a commercially available powdered grass product, as food and substrate are presented.

Keywords — Copper Larval instars *Chironomus tentans* Adult emergence Midge

INTRODUCTION

Chironomus tentans is a common midge species [1] that has been used as a test organism in the laboratory [2,3], and in laboratory and field contaminated-sediment studies [4–7]. Its large size and bright red color make it easy to work with, especially when surviving animals must be screened from sediments after testing. Midges are ubiquitous, abundant and important in nearly all freshwater and some marine environments [8,9]. Pollution tolerance varies among species; some resist relatively high levels of heavy metal pollution [6] only to succumb to extremely low levels of some toxic organic chemicals [10,11]. *C. tentans* is relatively tolerant to copper, compared to crustaceans and fish species, but is still killed by less than 1 ppm copper. Midge sensitivity is probably typical of insect species, but few others have been tested [12].

This study was designed to (a) provide information on the relative sensitivities of the life stages of *C. tentans* Fabricius (Diptera: Chironomidae) to copper, (b) determine the suitability of the different life stages for use in contaminated-water and sediment tests and (c) demonstrate unique flow-through rearing methods for the species.

METHODS AND MATERIALS

Rearing Methods

A simple and inexpensive procedure was used for the rearing and continuous culture of *C. tentans* in flow-through conditions, using the commercially available powdered

*To whom correspondence may be addressed.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

rye-grass product Cerophyl® (Agri-Tech, Inc., Kansas City, MO).

Two *C. tentans* egg masses, < 24 h old, were placed in a 10-liter aquarium covered with an adult flight cage (Fig. 1), with water flowing through the aquarium at approximately 100 ml/min. The eggs hatched after 3 d, at which time Cerophyl was added for use by the larvae as food and substrate. Food is added only after hatching to prevent fungus growth, which may suffocate young larvae. Approximately 20 ml (dry volume) Cerophyl was added to 100 ml water in a 250-ml beaker, stirred and allowed to settle for 1 min. About 25 ml of the water containing the finer suspended material was poured into the aquarium containing the newly hatched larvae; the remainder was then given to the older larvae in other aquaria as needed. Just enough Cerophyl was given to larvae for them to build tubes, plus a light "dusting" of particles between tubes. After day 20, approximately 10 ml of the Cerophyl–water slurry was introduced into each aquarium every other day. Fungus growth developed on the surface of the Cerophyl if too much was added. This was not critical, however, since the developing fungal mat could be broken up by gentle stirring. If too little Cerophyl was available for food and substrate, exposed larvae would be found on the surface of other larval tubes and swimming in the water column; normally, they would not be seen outside their burrows. The buildup of undigestable material over the 30 d of the life cycle provided the large mass of the tube-building material required by the third- and fourth-instar larvae (Fig. 2).

Summertime photoperiod (16 h light:8 h dark) and a temperature near 20°C allowed good production of eggs, larvae and adults. Six aquaria were used (Fig. 1) and new egg masses were placed in clean aquaria once a week to maintain a continuous supply of animals of known age. By dumping, cleaning and sterilizing occupied aquaria as they were needed for new eggs and larvae, a recyclable system provided all ages of midge larvae in an uninterrupted cycle.

Fig. 1. Ten-liter aquaria containing larvae and pupae, with plastic covers and net flight cages retaining adults.

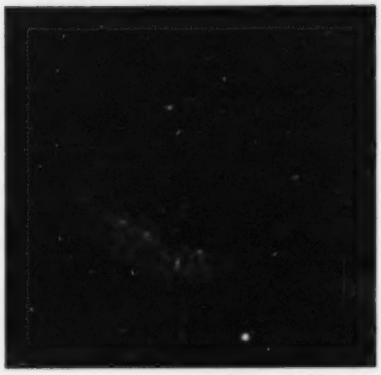

Fig. 2. Close-up showing the fourth-instar larval tubes and larvae.

Copper exposure test methods

Procedures outlined by the American Society for Testing and Materials [13] and the American Public Health Association [14] were used as basic guidelines. Analytical-grade copper chloride was the test chemical. Analysis for total copper was by atomic absorption spectrophotometry with direct aspiration or furnace techniques following U.S. Environmental Protection Agency–approved quality assurance procedures [14,15]. Test water was obtained from wells near the Willamette River, OR. Water quality varied depending on the time of year the tests were conducted, but tests with all larval stages in the same water type gave valid comparisons. A diluter [16] to deliver water containing uniform copper concentrations was used with the flow split to deliver test water to four 1-liter test beakers at each of five concentrations plus controls (Fig. 3). Screened overflows on the beakers prevented loss of test organisms (Fig. 4). Flow rate through the beakers was approximately 40 ml/min. Copper samples were taken at the start and end of the 96-h tests and more frequently during longer tests. In the one static-renewal test with first-instar larvae, plastic petri dishes were used as test containers, with water containing measured copper concentrations obtained from the diluter;

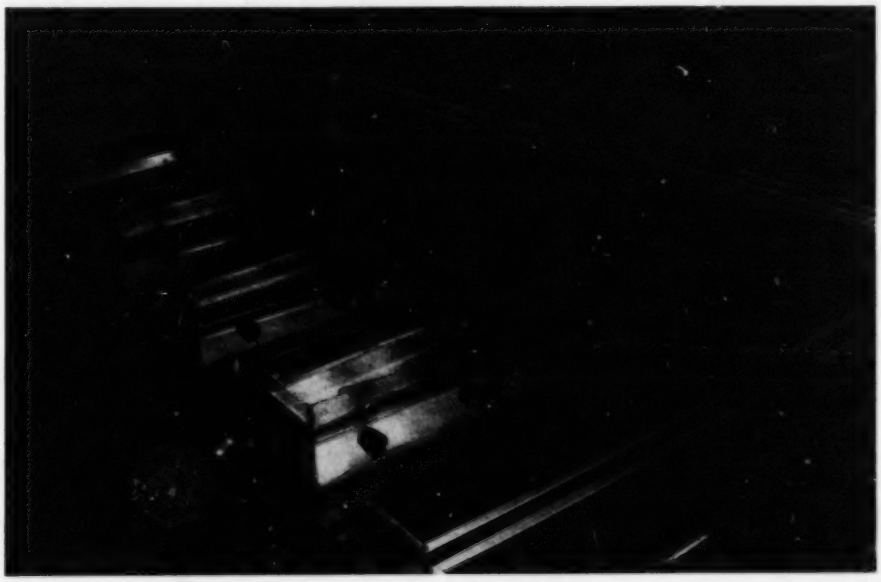

Fig. 3. Flow-through testing facility for the midge *C. tentans*, with five concentrations plus controls. Water flow is split to deliver test concentrations to four 1-liter test containers. The 20-liter aquaria containing the beakers also serve as water baths for temperature control.

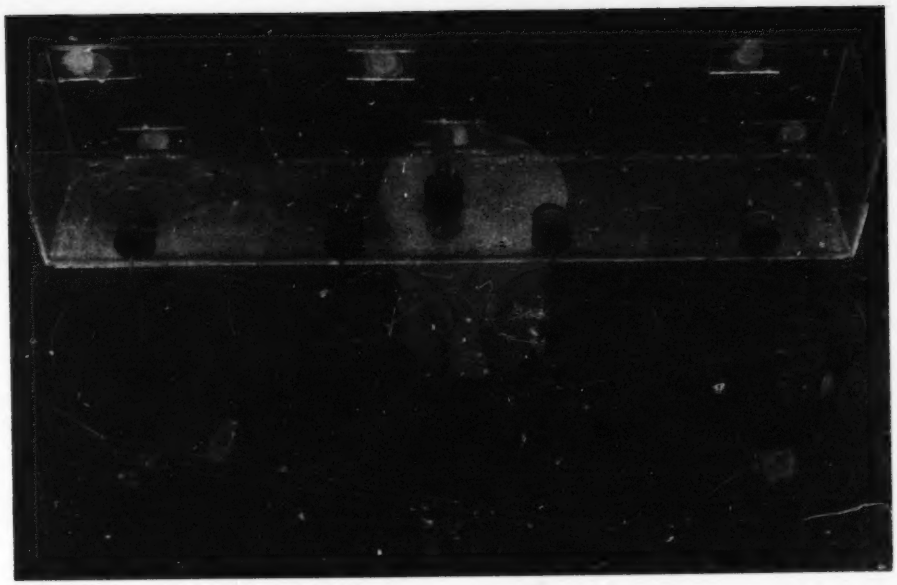

Fig. 4. Flow-splitting box with beakers, which are notched and have a screened overflow to prevent loss of test organisms.

the test water in the containers was renewed twice daily. Cerophyl was provided for food and substrate. Water temperature was continuously recorded, and fluorescent lighting over the test containers was set at a 16-h light:8-h dark photoperiod, with a light intensity of approximately 120 lux at the water surface. Water quality parameters monitored during testing were determined using standard methods [14]. Total alkalinity during testing varied from 60 to 67 mg/L (as $CaCO_3$) and pH averaged 7.4.

Tests were started with first-, second-, third- and fourth-instar larvae of known ages, with each life stage in separate containers. Equal numbers of larvae of each instar were placed in each 1-liter beaker or petri dish, with sufficient Cerophyl for them to construct burrows during exposure. First-instar larvae were tested by starting with one egg mass containing newly hatched larvae. The mass was cut into pieces, each containing approximately the same number of larvae, and one piece was placed in each petri dish. Older larvae tested in beakers were transferred and counted using a glass pipette to minimize handling injury. They were then exposed to five copper concentrations for 48 h, 96 h, 12 d or 20 d, depending on the test. Living and dead larvae, pupae or emerged adults were counted at the end of the test period and LC_{50} values were determined using methods of Hamilton et al. [17].

RESULTS AND DISCUSSION

First-instar larvae in initial tests with 1,000-ml beakers in the flow-through system (Tables 1 and 2) were difficult to observe or recover due to their small size (approximately 1 mm long), lack of pigmentation and their planktonic habit, which caused them to get caught in the surface film and on the overflow drain screens of the beakers. The static-renewal test conducted in petri dishes instead of 1,000-ml beakers (Table 3) produced useful information, primarily because the larvae seemed to go directly from the egg mass to the Cerophyl, and because the petri dish could be placed under a dissecting microscope to count surviving larvae.

Table 1. Results of test with three larval instars of *C. tentans* exposed to copper chloride for 48 h (19°C; water hardness 26 mg/L)

Measured copper concn. (μg/L)	No. of animals per beaker[a]	Percent survival at 48 h		
		First instar[a]	Second instar	Fourth instar
1,266	12	—	50	75
714	12	—	33	83
442	12	—	67	83
290	12	—	67	83
196	12	—	75	92
0	12	—	100	100

[a]25 first-instar larvae at each concentration. We were unsuccessful in locating and accurately counting survivors in Cerophyl substrate due to their small size.

Table 2. Results of test with the four larval instars of *C. tentans* exposed to copper chloride for 96 h (19.5°C; water hardness 84 mg/L)

Measured copper concn. (μg/L)	No. of animals per beaker	Percent survival at 96 h			
		First instar[a]	Second instar	Third instar	Fourth instar
1,900	10	—	30	40	40
1,100	10	—	50	60	90
600	10	—	50	60	90
400	10	—	60	60	80
300	10	—	70	90	100
0	10	—	100	100	100

[a]Many first-instar larvae were caught in surface film and died; dash indicates that none were counted.

Table 3. Results of test with first- and third-instar larvae of *C. tentans* exposed to copper chloride for 96 h in static and flow-through tests (20°C; water hardness 71 mg/L)

Measured copper concn. (μg/L)	No. of animals per chamber		Percent Survival at 96 h	
	First instar[a]	Third instar	First instar	Third instar
1,200	137	10	27	60
680	55	10	56	90
420	158	10	38	90
260	113	10	28	100
160	66	10	86	100
Control	99	10	83	100

[a]Started with one egg mass containing newly hatched larvae. The mass was cut up into pieces each containing approximately the same number of larvae and one piece was placed in each petri dish.

Table 4. Percent survival of fourth-instar larvae, percent completing pupal development and percent successfully emerging as adults for *C. tentans* exposed to copper chloride for 20 d at 15°C (water hardness 36 mg/L)

Measured copper concn. (μg/L)	No. of animals per beaker	96-h survival (%)	20-d survival (%)	Completed pupae development (%)	Adult emergence (%)
467	10	90	0	0	0
235	10	100	20	20	20
135	10	100	20	20	20
84	10	100	30	30	30
34	10	100	60	60	60
0	10	100	60	60	60

Table 5. Percent survival of *C. tentans* exposed to copper chloride for 12 d (19°C; water hardness 26 mg/L)

Measured copper concn. (μg/L)	No. of animals per beaker	Percent survival at 12 d		
		Fourth instar	Pupae	Adults[a]
529	10	70	0	0
278	10	70	0	0
175	10	60	20	0
129	10	80	10	0
76	10	50	20	0
0	10	80	60	20

[a]Test showing delayed development at higher concentrations was inadvertently terminated before all pupae completed development and successful adult emergence occurred.

Table 6. LC_{50} values calculated for *C. tentans* larval instars from measured copper concentrations

Instar[a]	Test duration	LC_{50} (μg/L)	95% Confidence limits	Water hardness (mg/L as $CaCO_3$)	Water temp. (°C)
First	96 h	298	242–366	71	20
Second	48 h	608	406–911	26	19
Second	96 h	773	373–1,559	84	20
Third	96 h	>1,200	—	71	20
Third	96 h	1,446	744–2,631	84	20
Fourth	48 h	>1,266	—	26	19
Fourth	12 d	> 529	—	30	19
Fourth	96 h	> 467	—	36	15
Fourth	96 h	1,690	1,264–2,259	84	20
Fourth	20 d	77.5	45–132	36	15
Fourth to adult emergence	20 d	77.5	45–132	36	15

[a]Test water obtained from diluter at measured concentrations and placed in plastic petri dishes for first-instar exposure.

The 96-h LC_{50} for the first-instar larvae was 298 μg/L, with narrow confidence limits, at a water hardness of 71 mg/L (as $CaCO_3$), based on measured total copper concentrations in water taken from the diluter and placed in the petri dishes. Since Cerophyl does complex copper, the LC_{50} values would probably be lower in static tests in which it was not used; however, substrate and food are required by the test animals for survival and growth. This should be considered when determining LC_{50} values. Cerophyl did not affect copper concentrations in the flow-through tests.

Second-instar larvae were apparently more tolerant of copper than first-instar larvae. A 96-h LC_{50} of 773 μg/L was determined from measured copper concentrations in the diluter at a water hardness of 84 mg/L. Third-instar larvae were more tolerant than second-instar larvae, with a 96-h LC_{50} of 1,446 μg/L in the same test water. The confidence limits for LC_{50} values with second-, third- and fourth-instar larvae were wide and overlapping, indicating that additional tests might produce different values.

Fourth-instar larvae were the most tolerant life stage tested, with a 96-h LC_{50} of 1,690 μg/L copper in the same test water having a hardness of 84 mg/L. Fourth-instar larvae that survived 20 d of exposure to copper (Table 4) pupated and emerged successfully as adults. A longer test substantially reduced the LC_{50} value from that obtained in 48-h or 96-h exposures (Tables 5 and 6).

From these tests, the comparative copper tolerance of the larval stages appeared to be fourth instar > third instar > second instar > first instar. At present, data obtained from tests with first-instar larvae may not always be reliable because of difficulties in conducting satisfactory tests, owing to the small size and planktonic habit of the larvae. The fourth instar was the most tolerant of the larval stages tested; unfortunately, this stage is used more frequently in tests than are other larval instars because of its larger size. Because the sensitive first-instar larvae are difficult to use in water or sediment assays, and fourth-instar larvae are relatively tolerant, the second-instar larvae appear to be the most suitable for acute toxicity studies. Larvae that are about 10 d old, when reared at 20°C, should be easy to use. If the test is started with older second-instar larvae, one or two molts will be included during a 96-h or 10-d test. At the end of the test, third- or fourth-instar larvae that are easy to recover will be found in control and nontoxic test containers.

The length of the testing period, as well as the life stage exposed, has a measureable effect on the results of the test, as the LC_{50} values in Table 6 illustrate. Test length appears to be as important, or possibly more important, than life stage in determining LC_{50} values.

CONCLUSIONS

The 10-d-old second-instar larvae were found to be most suitable for use in starting tests. They are large enough to work with and allow at least one larval molt during a 96-h acute test. They are the most sensitive of the three larger larval instars, are easy to handle and collect from rearing chambers and usually can be recoverd from sediments after exposures are completed.

Acknowledgement — We wish to thank W. Griffis and R. Titus for assistance with copper analyses, and S. Onjukka for assistance with testing and data analysis.

REFERENCES

1. **Sadler, W.O.** 1935. Biology of the midge *Chironomus tentans* Fabricius, and method for its propagation. Cornell University Agricultural Experiment Station Memoir 173, pp. 1–25.
2. **Dodge, E.F.** and **T.L. Theis**. 1979. Effect of chemical speciation on the uptake of copper by *Chironomus tentans*. *Environ. Sci. Technol.* **13**:1287–1288.
3. **Kawatski, J.A., V.K. Dawson** and **M.L. Reuvers**. 1974. Effect of TFM and Bayer 73 on in vivo oxygen consumption of the aquatic midge *Chironomus tentans*. *Trans. Am. Fish. Soc.* **103**: 551–556.
4. **Wentsel, R., A. McIntosh** and **W.P. McCafferty**. 1978. Emergence of the midge *Chironomus tentans* when exposed to heavy metal contaminated sediment. *Hydrobiologia* **57**:195–196.
5. **Cushman, R.M.** and **M.I. McKamey**. 1981. A *Chironomus tentans* bioassay for testing synthetic fuel products and effluents, with data on acridine and quinoline. *Bull. Environ. Contam. Toxicol.* **26**:601–605.
6. **Wentsel, R.** and **A. McIntosh**. 1977. Sediment contamination and benthic macroinvertebrate distribution in a metal-impacted lake. *Environ. Poll.* **14**:187–193.
7. **Kraft, K.J.** and **R.H. Sypniewski**. 1981. Effect of sediment copper on the distribution of benthic macroinvertebrates in the Keweenaw waterway. *J. Great Lakes Res.* 7:258–263.
8. **Anderson, R.L.** 1980. Chironomid toxicity tests — Biological background and procedures. In A.L. Buikema, Jr., and John Cairns, Jr., eds., *Aquatic Invertebrate Bioassays*. ASTM STP 715. American Society for Testing and Materials, Philadelphia, PA, pp. 70–80.
9. **Knight, A.W.** and **F. Knowlton-Dawley**. 1981. The biology and culture of Chironomus (sp.). Water Science and Engineering Paper 4514. Department of Land, Air, and Water Resources, University of California, Davis, pp. 1–62.
10. **Nebeker, A.V.** and **F.A. Puglisi**. 1974. Effect of polychlorinated biphenyls (PCB's) on survival and reproduction of *Daphnia, Gammarus,* and *Tanytarsus*. *Trans. Am. Fish. Soc.* **103**:722–728.
11. **Macek, K.J., K.S. Buxton, S.K. Derr, J.W. Dean** and **S. Sauter**. 1976. Chronic toxicity of lindane to selected aquatic invertebrates and fishes. EPA-600/3-76-046. U.S. Environmental Protection Agency, Duluth, MN, pp. 1–50.
12. **U.S. Environmental Protection Agency (EPA)**. 1980. Ambient water quality criteria for copper. EPA 440/5-80-036. Washington, DC.
13. **American Society for Testing and Materials (ASTM)**. 1980. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. ASTM Standard E 729-80. American Society for Testing and Materials, Philadelphia, PA. pp. 1–25.
14. **American Public Health Association, American Water Works Association, Water Pollution Control Federation (APHA)**. 1980. *Standard Methods for the Examination of Water and Wastewater* (and Supplement, 1981). Selected analytical methods approved and cited by the United States Environmental Protection Agency, 15th ed. Washington, DC.
15. **U.S. Environmental Protection Agency (EPA)**. 1979. Methods for chemical analysis of water and wastes. EPA-600/4-79-020. Cincinnati, OH.
16. **Garton, R.R.** 1980. A simple continuous-flow toxicant delivery system. *Water Res.* **14**:227–230.
17. **Hamilton, M.A., R.C. Russo** and **R.V. Thurston**. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. *Environ. Sci. Technol.* **11**:714–719. Correction 12:417 (1978).